Genetic Analysis of Resistance to Lettuce Drop Caused by *Sclerotinia minor*

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Abstract

Despite extensive germplasm screening, no lettuce accessions have been identified as possessing immunity to infection by *Sclerotinia* species. As previously reported, several genotypes have consistently shown a significant reduction in disease incidence compared with susceptible varieties following inoculation with *S. minor*. Many of these genotypes exhibit architectural features that may promote avoidance or escape from infection, such as upright growth and early bolting. To date, the genetic basis and mechanisms of resistance identified in lettuce remain unknown. Transfer of resistance that is due solely to avoidance into commercial cultivars without simultaneous transfer of unacceptable plant morphology may be difficult or impossible. In contrast, physiological resistance is likely to be more easily incorporated into acceptable cultivars. To facilitate the development of lettuce cultivars with *S. minor* resistance, we sought to ascertain the genetic basis of resistance from the primitive *L. sativa* accession PI 251246. Recombinant-inbred lines (RILs) were developed from a 'Salinas' x PI 251246 F_2 population to determine the heritability and action of genes involved in resistance derived from PI 251246 and for mapping of quantitative resistance loci. Results and implications of preliminary evaluation of $F_{2:4}$ RILs in a replicated field trial will be discussed.

INTRODUCTION

The disease lettuce drop is caused by two fungal species, *Sclerotinia minor* and *S. sclerotiorum,* and causes the complete collapse and soft rot of infected plants. Crop losses are routinely low to moderate in all production areas, and sporadically are very severe. Complete control is not acheived through cultural practices and fungicide applications, making resistant cultivars a top priority for the lettuce industry (Subbarao, 1998). Several lettuce (*Lactuca sativa)* cultivars and accessions have been described as partially resistant to either *S. minor* or *S. sclerotiorum* (Chupp and Sherf, 1960; Elia and Piglionica, 1964; Newton and Sequeira 1972; Madjid et al., 1983; Subbarao, 1998; Whipps et al., 2002). Since that time, partially resistant crisphead breeding lines have been developed (E.J. Ryder, pers. commun.), but commercial cultivars with an adequate level of resistance to lettuce drop are not available. This limited progress has been attributed to both the genetic complexity of and difficulties in assessing resistance.

S. minor usually infects the plant at the crown via mycelia following germination of soilborne resting structures (sclerotia). Resistance to *S. minor* has been identified and confirmed among a diverse array of lettuce genotypes, using both field and greenhouse evaluation methods (Grube and Ryder, unpublished results). Although no lines have shown immunity when using these procedures, several genotypes showed lower incidence of disease (DI) than susceptible controls. Germination of sclerotia, and therefore DI, is highly influenced by environment (Imolehin et al., 1980; Pennypacker and Risius, 1999). As a result, variability in DI observed in different experiments is common and can obscure differences between partially resistant and susceptible genotypes. Accurate selection of resistance therefore requires time-consuming and laborious replication within and repetition of tests.

To date, neither the inheritance nor the mechanism (s) of *S. minor* resistance identified in lettuce are known. Studies have suggested that resistance to *S. sclerotiorum* is under genetic control, but the specific genetic basis has not been examined (Newton and Sequiera, 1972). Our objective was to determine the feasibility of conducting genetic analyses of *S. minor* resistance using a RIL population developed by crossing the highly tolerant primitive *L. sativa* accession PI 251246 with the susceptible crisphead cultivar Salinas. We also sought to obtain preliminary information about possible mechanisms of resistance of PI 251246 by determining whether certain morphological traits showed an absolute association with resistance in the population examined. In this manuscript, we report results of field evaluation of $F_{2:4}$ RILs from the population described above, and discuss implications for future experiments.

MATERIALS AND METHODS

Plant Material

A recombinant-inbred line (RIL) population was created by crossing the *S. minor* – tolerant primitive *L. sativa* accession PI 251246 with the susceptible crisphead cultivar Salinas. Hybridity of F_1 plants was confirmed by morphological markers. Generations were advanced from the F_2 to F_5 generation by single-seed descent in the greenhouse in Salinas, Calif. Forty-seven F_4 RILs were evaluated for *S. minor* resistance and several morphological traits in a field plot at the USDA-ARS research station in Salinas, Calif.

Pathogen

High levels of *S. minor* were established in the experimental plot through a combination of continuous cropping with lettuce and the incorporation of additional *S. minor*-colonized rye seeds every 1 to 3 years for several years. Endemic inoculum was presumed to be a mixture of isolates. Supplemental inoculum was *S. minor* isolate 'Sm18', which was isolated from a lettuce field in Santa Maria, CA in 1993. To produce inoculum, rye seeds were mixed with water (1:1, v/v), autoclaved twice for 20 min, inoculated with mycelial plugs taken from the growing margin of 2-day-old potato dextrose agar (PDA) cultures, and incubated for 21 days at 20ºC with a 14-hour photoperiod. One to two *S. minor*-colonized rye seeds were placed 1 to 2 cm from the base of each plant approximately four weeks after transplanting.

Plot Layout

Lettuce seeds were sown in the greenhouse in plug trays. Four-week old seedlings were transplanted into the field in two rows on 1 m wide beds with 30 cm spacing. Treatments were randomly assigned to experimental units in four replicates in a randomized complete block design. Each experimental unit consisted of a 3 m plot containing 20 plants.

Trait Evaluation

Total plant number was counted at the time of inoculation. Plants were monitored for the appearance of lettuce drop symptoms at regular intervals throughout the field season. Final disease incidence (DI = proportion of plants killed) was determined 28 days post-inoculation (dpi).

Two of the four plots of each genotype were evaluated for one qualitative (leaf color) and several quantative traits. For all traits, the ratings obtained for the two plots evaluated were averaged. To permit timely evaluation, all traits except early bolting were evaluated categorically as follows: Leaf Surface – smooth = 0, intermediate = 1-2, blistered = 3; Heading Tendency – none = 0, slight = 1, strong = 2; Leaf Color – green = 0, segregating = 1, red = 2; Growth Habit – flat = 1, intermediate = 2, erect = 3; Axillary Branching – none = 0, intermediate = 1-4, strong = 5; Leaf Shape – narrow = 1, intermediate = 2-3, wide = 4; and Plant Diameter – small = 1, intermediate = 2-4, large = 5. For heterogeneous lines, the most extreme phenotypic values observed were averaged

to obtain a whole-plot rating for each segregating trait. The proportion of plants that had bolted by 28 dpi was also determined for each plot.

Statistical Analyses

All statistical analyses were carried out using JMP v. 4.0.2 (SAS Institute, Cary, NC). Pearson product-moment correlations were calculated using the multivariate platform and analyses of variance (ANOVA) were performed using the general linear model procedure. Significant differences between genotypes were calculated using Dunnett's test for comparing multiple treatments to a control, using parent genotypes as controls. Prior to ANOVA testing, DI data were evaluated for normality and were subjected to the arcsine transformation for binomial proportions with Bartlett's correction for proportions of zero and one (Snedecor and Cochran, 1989). Throughout this report, the term 'disease rating' (DR) refers to the transformed data, which was calculated as follows: arcsine ($\sqrt{p}$), where p = (total number of dead plants)/(total number of inoculated plants).

Broad-sense heritability was calculated as by Ben-Chaim and Paran (2000). Briefly, h^2_{bs} = (Vseg-Vnonseg)/Vseg, where Vnonseg = the environmental variance estimated for the non-segregating generations (parent genotypes) and Vseg = that estimated for the segregating population (RILs). Environmental variance estimates were MSE values obtained by ANOVA.

RESULTS

S. minor **Resistance**

The incidence of lettuce drop was moderate in the experimental plot, with the susceptible cultivar Salinas having an average disease rating (DR) of 53.44 by 28 dpi. The RILs and parents showed a wide range of responses, with DR ranging between 6.79 and 68.68 (Fig. 1). A significant portion of the observed variability in DR was attributed to genotype (Table 1). The resistant parent PI 251246 (DR = 12.12) and 21 of the $F_{2:4}$ RILs had significantly lower DR than that observed for 'Salinas' (Dunnett's test, $\alpha = 0.05$).

Mean DR values of the RILs were normally distributed (Shapiro-Wilk Test, p = 0.3670), with overall mean DR of 34.21. Seven RILs had DR values less than PI 251246 and four RILs had DR greater than 'Salinas', although none of these differences were statistically significant. The broad-sense heritability calculated for DR was high (h^2_{bs} = 0.918).

Morphological Traits

While most lines were homogeneous for most traits, some variation was observed within lines. Histograms depicting the frequency distributions of average trait values for $F_{2:4}$ lines are shown in Fig. 2. The trait values of the parent genotypes are shown with arrows on each histogram. Phenotypic variability among RILs was observed for all of the traits measured, with some lines showing the full range of values up to and including parental values for most traits. The exception was heading tendency; no $F_{2:4}$ lines showed as strong a tendency to form heads as 'Salinas'. Transgressive segregation was observed for several traits. For example, some lines exhibited more blistered leaves, flatter growth habit, larger frame size, and more axillary branching than was observed in either parent genotype.

Red leaf coloration in lettuce is caused by the presence of anthocyanin, which requires the action of two complementary dominant alleles, 'C' and 'G', at unlinked loci. Both were present in the red parent, 'PI 251426', and both were absent in the green parent, 'Salinas'. Among the 47 $F_{2:4}$ lines, the observed segregation for leaf color (10 red: 14 segregating: 23 green) was consistent with that predicted by Mendelian segregation (6.6 red: 11.8 segregating: 28.6 green) of these alleles (χ^2 = 3.280, p = 0.19).

Correlations

Significant correlations were observed between some morphological traits (Table 2). Early bolting was associated with erect growth habit, narrow leaf shape, smoother

leaves, and increased axillary branching. Wider leaves and a less erect growth habit were associated with increased heading tendency, and an erect growth habit was also associated with reduced leaf blistering.

Resistance to *S. minor* was significantly associated with several traits (Table 2). Lower DR was most strongly associated with an erect growth habit, and was also associated with early bolting and narrow leaf shape. Leaf blistering and increased heading tendency were associated with higher DR, or increased susceptibility. These correlations were not absolute. For example, of the 21 RILs that had significantly lower DR than 'Salinas', two had no bolted plants by 29 dpi, two others had very flat growth habits (mean < 1.75), and five exhibited at least some heading tendency.

DISCUSSION

Our results suggest that there is sufficient measurable genetic variation to permit genetic analysis of resistance to *S. minor* in the PI 251246 x 'Salinas' population. Disease ratings of the $F_{2:4}$ RILs evaluated were normally distributed, suggesting quantitative inheritance. The heritability estimate obtained in this experiment was high, suggesting that, despite genetic complexity, gain from selection of resistance should be possible. Variability between experiments, which has been frequently reported by researchers working with all *Sclerotinia* species (Abawi et al., 1980; Pennypacker and Risius, 1999), would lower the estimated heritability and could account for the limited tangible results from resistance breeding efforts. Since our estimate is based on only a single field experiment, it is likely that we have overestimated the heritability of resistance. Evaluation of advanced RILs in additional tests and environments is necessary to permit more accurate determination.

In other crop plants, two types of resistance to *S. sclerotiorum* have been described: 1) physiological resistance and 2) escape mechanisms that minimize the opportunity for infection (Mestries et al., 1998; Arahana et al., 2001; Miklas et al., 2001; Park et al., 2001). To the authors' knowledge, mechanisms of resistance to *S. minor* have not been studied. We hypothesized that avoidance could be the primary mechanism of resistance of PI 251246, which has an early-bolting upright growth habit which minimizes contact between soft plant tissue and the soil surface, where infectious propagules reside. Since the parents of the population differed for several morphological characters, the segregation of several traits among the RILs allows us to directly test our hypothesis. The two known major genes that were polymorphic in this population (C and G) segregated in a manner consistent with Mendelian inheritance, providing no evidence for segregation abnormalities in this population that could affect subsequent genetic analyses. To evaluate segregation distortion in a systematic way, analysis of markers distributed evenly throughout the genome will be required.

For the families evaluated, resistance to *S. minor* was significantly associated with several features of PI 251246, including an erect growth habit, early bolting, narrow and smoother leaves, and reduced heading tendency. Due to the probable polygenic nature of resistance, some associations between resistance loci and other loci from PI 251246 are expected due to linkage. The fact that resistance was not absolutely associated with any particular morphological trait suggests that, while escape mechanisms may play a role in resistance, PI 251246 also appears to possess physiological resistance. This suggests that it will be possible to develop horticulturally acceptable (slow-bolting, heading) cultivars with higher levels of partial resistance from PI 251246.

In conclusion, we have shown that genetic analysis of resistance to *S. minor* should be feasible in the RILs being developed, and have presented results suggesting that several possible mechanisms may be involved in determining the resistance of PI 251246. To determine the inheritance and mechanisms of *S. minor* resistance in lettuce, subsequent evaluation of increased numbers of RILs of advanced generations in multiple test sites and locations, using both field and greenhouse resistance evaluation methods, will be required. The preliminary results presented indicate that further investment in this approach is warranted.

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Tables

Table 1. Analysis of variance of disease rating (DR) for $F_{2:4}$ RILs and parent genotypes in replicated field trial.

Source	DF	Sum of Squares	Mean Square	F ratio	P
Model	51	37311.55	731.60	8.41	<0.0001
Genotyp	48	36321.49	756.70	8.70	<0.0001
Replicate	3	990.06	33.02	3.80	0.0118
Error	144	12524.97	86.98		
Total	195				

Table 2. Correlations[1] between lettuce drop disease ratings and several horticultural traits among $F_{2:4}$ RILs.

	DR[2]	EB	LS	AB	LC	Head	Blister
Habit	-0.64**	0.52**	-0.34*	-0.05	-0.13	-0.32*	-0.43**
Blister	0.46**	-0.42**	0.42**	-0.25	-0.28	-0.11	
Head	0.34*	-0.18	0.31*	0.23	0.02		
LC	0.03	0.06	-0.06	0.04			
AB	0.11	0.40**	-0.13				
LS	0.54**	-0.62**					
EB	-0.53**						

[1] Significance at the 0.05 and 0.01 probability levels are denoted by * and **, respectively.
[2] Abbreviations for traits measured are as follows: disease rating (DR), early bolting (EB), leaf shape (LS), axillary branching (AB), leaf color (LC), heading tendency (Head), leaf surface (Blister), and growth habit (Habit).
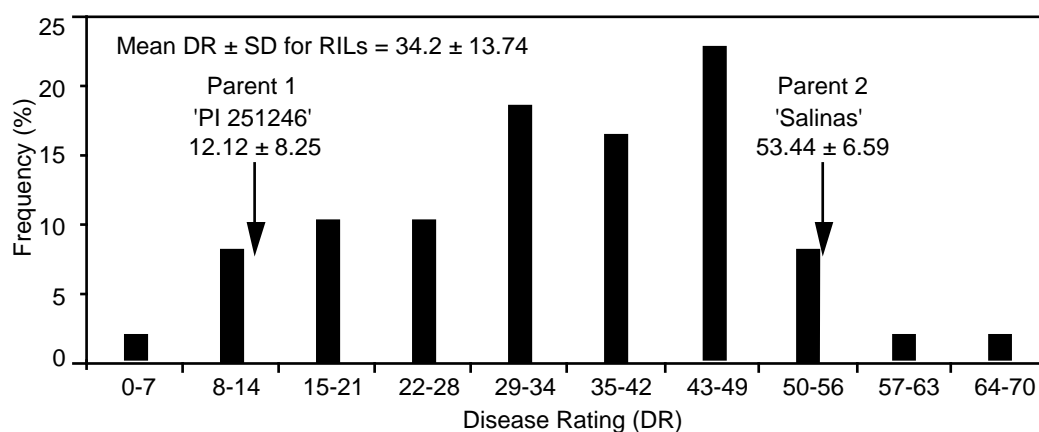
Figures

Fig. 1. Frequency distribution of the mean lettuce drop disease ratings for 47 $F_{2:4}$ lines and parents in a single year and test site.
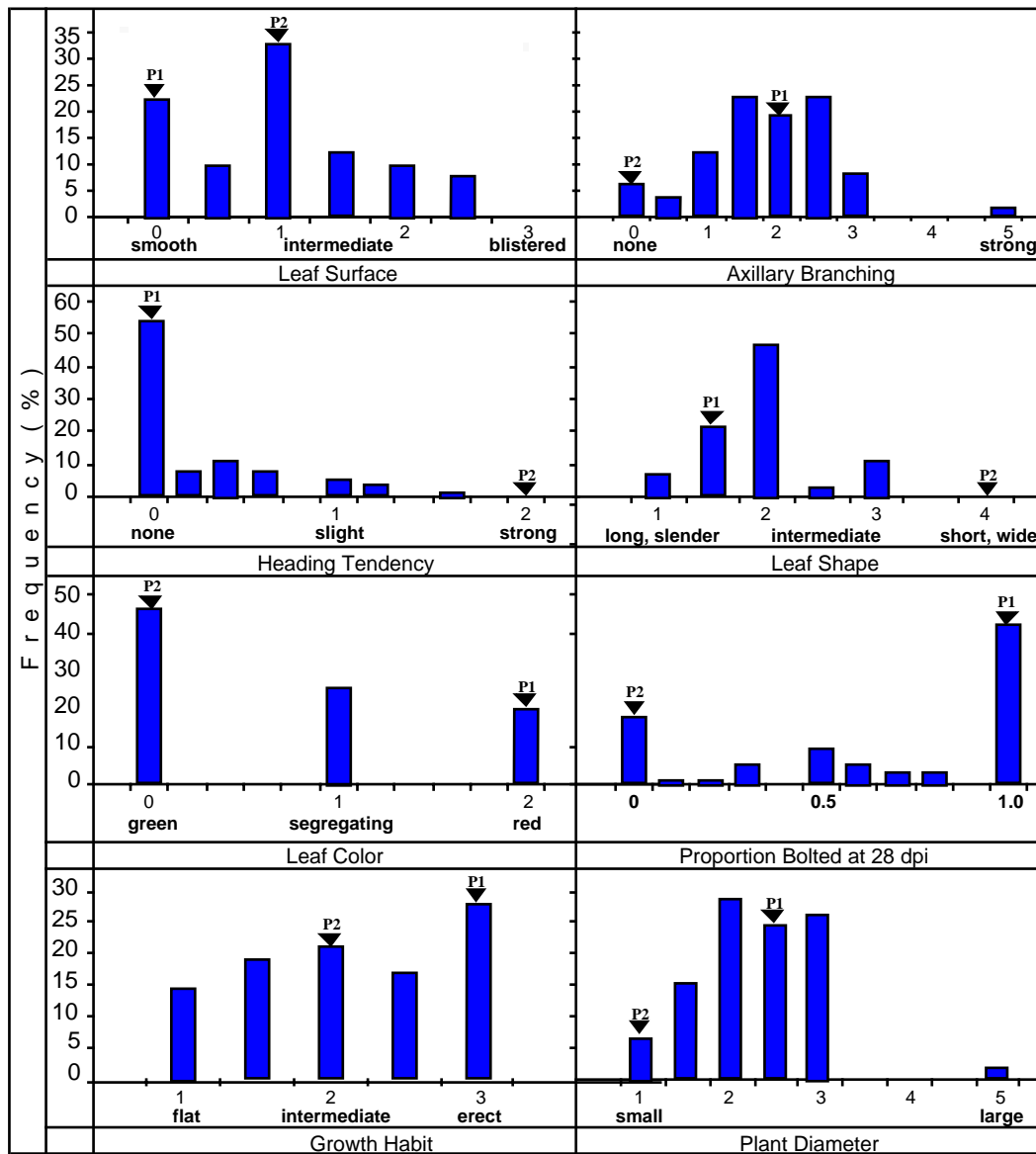
Fig. 2. Frequency distribution of mean ratings for horticultural traits evaluated for 47 $F_{2:4}$ lines and parents in a single year and test site.